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A Slide Antigen in the Indirect Fluorescent Antibody Test for *Trichinella spiralis**

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Summary. A stable cuticular slide antigen was prepared for use in the indirect fluorescent antibody (IFA) test for trichinosis. Cuticles of *Trichinella spiralis* larvae, prepared by pepsin digestion, were embedded in Tissue-Tek OCT embedding medium, frozen, and sections made and mounted on slides. After storage at −70° for 2 days to allow the antigen sections to become firmly affixed to the slides, they may be used to perform the IFA test for trichinosis. This method is less time-consuming, due to the elimination of centrifugation steps, than the antigen presently in use for the trichinosis IFA test, and should be applicable to other IFA procedures.

INTRODUCTION

Fluorescent antibody methods have been employed for the study of *Trichinella spiralis* by many investigators. In addition to a better understanding of the immune response of the host, these investigations have led to the development of an indirect fluorescent antibody (IFA) test for antibodies to *T. spiralis*.

In an early study, Jackson (1959) reported that precipitates formed around anal and oral orifices of living *T. spiralis* larvae stained brightly after exposure to antisera labelled with fluorescein isothiocynate. Labzoffsky, Kuitenen, Morrissey and Hamvas (1959) found that cuticles of *T. spiralis*, mechanically freed of internal organs, reacted specifically with antisera in fluorescent antibody tests. In 1962, Sadun, Anderson and Williams described an IFA test in which whole fixed larvae were used as antigen. This whole larvae antigen had a shelf life of only a few weeks. Sulzer (1965) showed that cuticles of *T. spiralis* larvae, with their internal organs removed by pepsin digestion could be used as antigen and that this antigen was relatively stable on storage.

The test as described by Sadun *et al.* (1962) and modified by Sulzer (1965) was performed in test tubes. It was shown by Sulzer and Kagan (1967) that multiple washes of sensitized antigen were required if a prozone-like reaction, described by Sadun (1963), was to be avoided.

To circumvent repetitive washings and render the test less cumbersome, Kozar, Karmanska, and Kozar (1966) fixed the cuticular antigen to slides by heat. However, Kozar (1967) found that this antigen was too sensitive. He, therefore, returned to the tube method of performing the test. The advantages of antigen particles fixed to slides were so

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great that we also attempted to develop a slide antigen. A preliminary evaluation of the procedure that we found successful follows.

MATERIALS AND METHODS

Cuticles of *T. spiralis* larvae were prepared as reported by Sulzer (1965). Larvae, freed from host tissue by pepsin digestion, were digested in fresh pepsin solution for an additional 36–40 hours. Care was taken to terminate the digestion of the larvae at the point when all larvae still contained some of the residual internal structures. These cuticles were concentrated in a small amount of fluid and mixed with Tissue-Tek OCT embedding medium *(Ames Co.)[1] in a one-to-one proportion. The mixture was carefully stirred so that the digested larvae were distributed evenly throughout the embedding medium.

The antigen-embedding fluid mixture was placed in Beem Plastic Embedding capsules (E. F. Fullman Co. No. 5026)[2]. It was convenient to prepare the mixture directly in the capsule, since the medium is very viscous. The capsules were capped and placed on a small piece of dry ice to solidify the embedding medium. They were then ready for the sectioning procedure.

The capsules were split along one side with a razor blade and removed from the frozen OCT specimen core. The core was placed pointed end down in an aluminium foil mold filled with OCT embedding medium; a labelled cryostat stem (chuck) was placed face down on top of the mould. This part of the procedure was carried out on dry ice to prevent the specimen from thawing. When the material in the mould had frozen, the aluminium was peeled off, and the specimen placed on the microtome in a cryostat adjusted to $-30°$.

Sections were cut at 10 μm thickness from the block; they were picked up on warm (room temperature) slides which had been previously smeared with Mayer's albumin adhesive. The slides were then placed in a slide box in the cryostat. When the box was filled, it was covered and kept in a freezer at $-70°$ until ready for processing for examination. When the desired number of sections were cut from the block, the remaining part was wrapped in aluminium foil and stored *en bloc* in a freezer for future use.

For evaluation of the antigen, a battery of forty-two sera was assembled; nineteen of these had positive reactions, and twenty-three negative reactions in the bentonite flocculation (BF) test (Bozicevick, Tobie, Thomas, Hoyem and Ward, 1951) for antibodies to *T. spiralis*. A four-fold dilution series which began at 1:5 was used to titrate these sera. Phosphate buffered saline (PBS), pH 7·2, was used as diluent and wash fluid.

All sera were first tested at dilutions of 1:5 and 1:20 for antibody activity. Those that gave positive reactions at 1:20 were diluted and tested to determine the end-point titre, defined as the greatest dilution at which specific green fluorescence of cuticular material could be seen. In negative slides only the *residual internal* organs, stained light pink by counterstain, were visible. In positive reactions, the cuticle surrounding the internal structures appeared as green veil-like material. After the first determination of IFA titres of the sera, sixteen of the positive reactors were retested in a 'blind' test, i.e. the test slides were assorted and coded before reading, to determine reproducibility of results.

The test procedure was as follows. Antigen slides were stored for at least 2 days in a $-70°$ freezer to allow the antigen particles to become firmly affixed to the slides. They were

(1) Ames Co., Division Miles Laboratories, Inc., Elkhart, Indiana.
(2) E. F. Fullam, Inc. P.O. Box 444, Schenectady, New York 12301, U.S.A.
* Use of trade names is for identification only and does not constitute endorsement by the Public Health Service or by the U.S. Department of Health, Education, and Welfare.

brought to room temperature and fixed for 30 minutes with 10 per cent formalin prepared in 0·5 per cent bovine serum albumin dissolved in PBS. They were air dried, labelled, and placed in a moist chamber. The serum dilutions were distributed on the appropriate slides. These slides were then incubated for 30 minutes at 37°, washed in PBS for 10 minutes, air dried, and replaced in the moist chamber. Each antigen mount was covered with a 1:20 dilution of fluorescein labelled anti-human conjugate to which Evan's blue, 1:1000, was added. The slides were again incubated at 37° for 15 minutes, and then washed for 10 minutes in PBS. After air drying, the mounts were covered with a drop of buffered glycerol, pH 8·0, and a cover slip added. An American Optical fluorescence microscope, equipped with a BG-12 exciter filter and a GG-9 ocular filter and illuminated by an Osram HBO-200 mercury arc burner, was used to evaluate the reaction.

RESULTS

Reactions of the sera in the IFA and bentonite flocculation (BF) tests are shown in Table 1. All nineteen sera that were positive in the BF test were also positive in the IFA test. In addition, four sera that were negative in the BF test were positive in the IFA test, two at 1:5, one at 1:20, and one at 1:80.

TABLE 1

ANTIBODY TITRES OBTAINED IN THE IFA TEST, USING CUTICULAR ANTIGEN ON SLIDES, AND TITRES OBTAINED IN THE BF TEST FOR *Trichinella spiralis*

BF titres	IFA titres						
	Negative	1:5	1:20	1:80	1:320	1:1280	1:5120
Negative	19	2	1	1	0	0	0
1:5	0	0	0	1	0	1	0
1:20	0	0	0	3	3	1	0
1:80	0	0	0	1	2	1	1
1:320	0	0	0	0	3	0	0
1:1280	0	0	0	0	1	1	0

Of the sera positive in both tests, thirteen had the same titre or differed by only one four-fold dilution. Four-fold differences are considered within the reproducibility of the two tests. Four of twenty-three sera from suspected cases of trichinosis, originally found negative in the BF test, gave positive reactions in the IFA test.

When sixteen of the positive sera were retested in the IFA test, all titres were reproduced within one four-fold dilution. Ten sera gave identical titres in the two tests, four sera were one dilution less, and two were one dilution higher than when tested the first time.

DISCUSSION

As shown in Table 1, no false negative reactions occurred with the slide antigen in the IFA test. Since all the sera in the test battery were from individuals suspected of having trichinosis, the four sera found positive for antibodies to *T. spiralis* in the IFA test, but were negative in the BF test may, in fact, be true positives. In their original evaluation of cuticular antigen, Sulzer and Chisholm (1966) found that the IFA test gave a statistically greater number of positives than the BF test in suspected cases. The enhanced sensitivity

of the IFA test may be due to the fact that the BF test is dependent on agglutinating antibodies, whereas the IFA test can react with any antibody, whether or not it has agglutinating or precipitating properties.

The greater reactivity, i.e. tendency to higher titres, of the IFA as compared with the BF test is illustrated in Table 1. Within the limits of reproducibility ($\pm$ one four-fold dilution), all of the titres in the IFA test are either the same as or greater than the titres obtained with the same sera in the BF test. This may also reflect the ability of the IFA test to detect antibodies that do not contribute to the agglutination reaction in the BF test. That is, if a major portion of the antibodies are not of the agglutinating type, this portion would account for the relatively greater IFA test titres.

Slide antigen prepared as described above generally behaves in the same manner as cuticular antigen used in tube tests. When sixteen sera positive in the IFA procedure were retested, no reactions varied more than four-fold from the first determination. Extreme sensitivity does not seem to be a factor with this slide antigen, but as noted above, sensitivity is greater than that of BF tests on the same sera.

The cuticular antigen fixed on slides has definite advantages over the cuticular antigen used in tube tests. The former requires only three washings whereas the latter requires six washes. Eliminating centrifugation at each washing step in the tube test further reduces the time for completing the test.

Experiments on prefixing the antigen prior to embedding and sectioning are being carried out. Shelf life studies are in progress, although there seems to be no reason why this antigen should be less stable than cuticular antigen particles stored in formol.

The technique of sectioning used for this slide antigen should be applicable to all the IFA tests now requiring processing of larval or other life stages of parasites as antigen, especially those in the size range of *T. spiralis*. The method is already being used by the authors in developing an IFA test for *Onchocerca volvulus*.

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